

SUNY Geneseo
KnightScholar

Psychology faculty/staff works

Department of Psychology

2002

Sexually dimorphic behavioral responses to prenatal dioxin exposure

R. Hojo

S. Stern

G. Zareba

V.P. Markowski

C. Cox

See next page for additional authors

Follow this and additional works at: <https://knight scholar.geneseo.edu/psychology-faculty>

Recommended Citation

Hojo R., Stern S., Zareba G., Markowski V.P., Cox C., Kost J.T., Weiss B. (2002). Environmental Health Perspectives, 110, 247-254 doi: 10.1289/ehp.02110247

This Article is brought to you for free and open access by the Department of Psychology at KnightScholar. It has been accepted for inclusion in Psychology faculty/staff works by an authorized administrator of KnightScholar. For more information, please contact KnightScholar@geneseo.edu.

Authors

R. Hojo, S. Stern, G. Zareba, V.P. Markowski, C. Cox, J.T. Kost, and B. Weiss

Sexually Dimorphic Behavioral Responses to Prenatal Dioxin Exposure

Rieko Hojo,¹ Sander Stern,¹ Grazyna Zareba,¹ Vincent P. Markowski,² Christopher Cox,³ James T. Kost,³ and Bernard Weiss¹

¹Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA;

²Department of Psychology, University of Southern Maine, Portland, Maine, USA; ³Department of Biostatistics, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA

Pregnant Sprague-Dawley rats received a single oral dose of 0, 20, 60, or 180 ng/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on day 8 of gestation. Each litter contributed a single male–female pair trained to press a lever to obtain food pellets under two operant behavior procedures. Initially, each lever press was reinforced. The fixed-ratio (FR) requirement was then increased every four sessions from the initial setting of 1 to values between 6 and 71. We then studied responses for 30 days under a multiple schedule combining FR 11 and another schedule requiring a pause of at least 10 sec between responses (DRL 10-sec). TCDD evoked a sexually dimorphic response pattern. Generally, TCDD-exposed males responded at lower rates than control males. In contrast, exposed females responded at higher rates than controls. Each response measure from the mult-FR DRL schedule yielded a male–female difference score. We used the differences in response rate to calculate benchmark doses based on the relative displacement from modeled zero-dose performance of the effective dose at 1% (ED₀₁) and 10% (ED₁₀), as determined by a second-order polynomial fit to the dose–effect function. For the male–female difference in FR rate of responding, the mean ED₁₀ was 2.77 ng/kg with a 95% lower bound of 1.81 ng/kg. The corresponding ED₀₁ was 0.27 ng/kg with a 95% lower bound of 0.18 ng/kg. For the male–female difference in DRL rate, the mean ED₁₀ was 2.97 ng/kg with a 95% lower bound of 2.02 ng/kg. The corresponding ED₀₁ was 0.30 ng/kg with a 95% lower bound of 0.20 ng/kg. These values fall close to, but below, current estimates of human body burdens of 13 ng/kg, based on TCDD toxic equivalents. **Key words:** behavioral toxicology, benchmark dose, neurobehavioral function, operant behavior, prenatal exposure, sexual dimorphism, TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 110:247–254 (2002). [Online 6 February 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p247-254hojo/abstract.html>

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype and most toxic member of a class of halogenated compounds, the polychlorinated dioxins (PCDDs), which are distributed widely in the environment. PCDDs are now recognized as potent developmental toxicants, provoking adverse effects in virtually every organ system studied. Public health concerns have been raised especially because of their ubiquitous presence in the environment and their retention in body tissues for extended periods. The half-life in humans of TCDD is in the range of 7–10 years. The developing organism may be particularly sensitive to TCDD exposure; some laboratory studies have reported that the fetus appeared to be 100-fold more sensitive than the adult (1). Because of its lipophilic properties, structural stability, and long half-life (2), TCDD stored in fat tissue is transferred via the placenta and maternal milk to developing offspring during gestation and lactation (3,4).

Disorders of sexual development are among the best-documented outcomes of prenatal exposure, and include genital abnormalities, impaired sexual performance, and reduced reproductive success. Some of these effects have occurred at levels in animals close to the human estimated background body burden of 13 ng/kg, as

calculated from the sum of TCDD equivalents (TEQs) (5). PCDDs are believed to exert their effects through a ligand-activated transcription factor, the aryl hydrocarbon receptor (AhR). AhR is expressed in most organs and cells in the body. A crucial role for AhR in development is shown by the numerous defects observed in transgenic mice lacking it (6,7).

Because of TCDD's effects on gonadal and thyroid hormone function (8,9), which are essential elements in brain development, it is also reasonable to assume that its actions will be reflected in neurobehavioral indices. The enormous TCDD literature, however, contains surprisingly little information on this topic.

Regarding behavior, only a handful of studies are available. Schantz and Bowman (10) conducted a pioneering study in monkeys exposed to TCDD prenatally. Although the exposed offspring displayed retarded learning of shape reversals, they performed equivalently to controls on spatial and color reversals. The dose administered to the pregnant monkeys (0.126 ng/kg/day) would have produced a body burden of 19 ng/kg, equivalent to the lowest dose used in our own studies and close to human background levels. In a later rat study (11), offspring whose mothers were exposed to a total of

175 or 700 ng/kg TCDD during gestation days (GDs) 10–16 showed decreases in error scores on a radial maze, particularly in males, but the exposed animals did not differ from controls on a delayed spatial alternation task based on a T-maze. Subsequent studies from Schantz's laboratory (12,13) also showed a change in spatial learning and memory in exposed male offspring. Although the change facilitated responding so that exposed animals performed more efficiently, the apparent improvement appears to be an artifact of the experimental contingencies (i.e., a TCDD-induced behavioral stereotypy would account for the changes). Learning deficits were seen in both sexes on a discrimination reversal learning task.

We recently reported that female offspring of Holtzman rats that had been exposed to a single oral dose of 0, 20, 60, 180 ng/kg of TCDD on GD 18, showed dose-related changes in behavior (14). In that study, the rats pressed a lever under a fixed-ratio schedule of reinforcement to obtain a 30-sec opportunity to run in a running wheel. Benchmark dose analyses located the mean ED₁₀ (effective dose at 10%) and BMD₁₀ (benchmark dose at 10%) for two measures of performance in a range between 7 and 10 ng/kg.

Schedule-controlled operant behavior (SCOB) provides a powerful tool for examining neurobehavioral function. In this class of behavioral procedures, a relationship is defined between the behavior of a subject and its consequences in a defined environment. SCOB provides numerous procedures for the analysis of learning, performance, and memory (15), as well as providing the ability to tailor tasks to model complex cognitive activities in humans. Under both transitional and steady-state conditions, SCOB studies have been used extensively to detect

Address correspondence to B. Weiss, Department of Environmental Medicine, Box EHSC, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 USA. Telephone: (716) 275-1736. Fax: (716) 256-2591. E-mail: bernard_weiss@urmc.rochester.edu

We gratefully acknowledge the assistance of J.W. Kai. TCDD was administered in the University of Rochester Environmental Health Sciences Center's Supertox Facility, directed by T. Gasiewicz.

This research was supported by National Institute of Environmental Health Sciences center grant ES012247 and by grant ES089858 to B. Weiss.

Received 23 May 2001; accepted 9 August 2001.

and interpret outcomes of exposures to pharmacologic and toxicologic agents (16), including developmental exposures (17,18).

In the present study, we examined schedule-controlled operant performances of male and female littermates whose dams had been administered TCDD on GD 8. Although GDs 9–10 in the rat represent the onset of organogenesis and brain development unfolds later (19), GD 8 was chosen for a number of reasons specific to TCDD. Abbott et al. (20) found in mouse embryos that AhR mRNA and protein were expressed in GD 10–13 neuroepithelium, and that, as development progressed, levels in brain decreased. Also, Abbott and Probst (21) found that GD 10–11 mouse embryos showed the highest levels of aryl hydrocarbon nuclear translocator (ARNT) in neuroepithelial cells of the neural tube. They did not evaluate ARNT at earlier times. If the toxic effects of TCDD are closely linked to AhR binding, it is critical to determine the consequences of exposure during the period of elevated AhR expression in accord with the concept that tissue concentration at the critical window of sensitivity is a key dose metric. Hurst et al. (22) administered a dose of 200 ng/kg to pregnant Long Evans rats on GD 8 and found that fetal TCDD levels were maximum (39.6 pg/g) on GD 9 and then fell slowly.

In the present study, we administered a single oral dose of 0, 20, 60, or 180 ng/kg TCDD to pregnant female rats on GD 8. We studied the behaviors of both male and female offspring under two different schedule-controlled, food-reinforced operant procedures: fixed ratio (FR) and multiple FR 11, differential reinforcement of low rate (DRL) 10 sec (mult-FR 11, DRL 10 sec). Two general hypotheses were examined: TCDD would alter the operant behavior; TCDD effects would depend partially on sex.

We studied the FR initially under an incremental FR condition. The FR schedule specifies that every n th response is reinforced. In the incremental FR condition, the FR value was increased every 4 days in an ascending series of values ranging between 1 and 71. Such a progression allowed us to study the transition-state performances (23,24) that occur in response to changes in experimental conditions. Transition-state performances are of particular interest because they reflect the ability of the subject to learn, adapt, or adjust to changing environmental circumstances. The rate and form of such behavioral adjustments may indicate an adverse effect not seen under final steady-state conditions during which compensatory factors have had an opportunity to emerge. Other investigators have also demonstrated the sensitivity of transition states, particularly

under FR schedules, in studies of prenatal or early developmental effects of ethanol (25,26), methyl and elemental mercury (27,28), cadmium (29), and lead (27,30,31). Our recent TCDD experiment (14), showed that running-wheel FR transition-state performances of rats were especially sensitive to gestational exposure to TCDD.

A transition-state procedure can also be viewed as a dynamic challenge that requires the subject to adjust to a new set of circumstances and may thereby reveal deficits or vulnerabilities not seen under steady-state conditions. Unmasking silent toxicity can be achieved using behavioral or other forms of challenges, such as pharmacologic agents (32) or conditions that impose stress on the subject. Such challenges have been used to reveal delayed neurotoxicity (33) after developmental exposures to neurotoxic agents, as well as to evaluate its mechanisms (34).

In a multiple schedule, two or more simple schedules of reinforcement are presented in successively alternating components, with unique stimulus conditions such as visual or auditory stimuli signaling which component is in effect. Typically, the performance of a well-trained rat in which good discriminative control has been established switches between the components so that responding in each component resembles that seen in a rat trained only under that specific schedule. A DRL component was combined with an FR component in the present study. Under a DRL schedule, a clock begins at the onset of the component and after each lever press. Only a press emitted after the specified interval (10 sec in this experiment) has elapsed is reinforced with a food pellet. If the rat responds too early, the clock is reset, and the 10-sec waiting period begins again. Under this contingency, then, lower rates of responding yield higher rates of pellet delivery. In contrast, high FR rates yield high rates of food delivery. In this experiment, we expected to see high rates of responding in the FR component and low rates in the DRL component. Performances under the DRL schedule, like those under the FR schedule, have proven sensitive to developmental neurotoxins (35).

A multiple schedule offers several advantages. First, by combining schedules of potentially different sensitivities to the exposure agent, we increase the likelihood of measuring exposure effects. Second, interpreting the nature of the toxicity may be facilitated by comparing the results across the component schedules (16). The results may assist in identifying nonspecific influences because, in a sense, one component schedule acts as a baseline control for the other; performance on the two components may suggest sensory deficits (36); they may implicate cognitive

processes involved in complex learning and memory; or they may suggest a role for a specific neurochemical involvement or other mechanisms of action (37).

Sex differences often emerge under SCOB contingencies. Because gonadal hormones can influence differences in responding between males and females in operant behaviors such as lever-pressing, neurotoxins that disturb the organizational effects of these hormones on brain development could potentially produce enduring performance changes (38). Should developmental TCDD exposure interfere with sexual differentiation of the brain, we would expect to observe an altered pattern of sex differences in behavior.

Normal male rats, for example, tend to emit higher overall response rates than females under ratio schedules (39) or under schedules that differentially reinforce high rates of responding (40), both of which appear to elicit a food-motivated function called behavioral perseverance. Male rats, in fact, display food-motivated perseverance across several behavioral manipulations. Male rats spend more time than females holding down a lever if holding is food reinforced (41). Male rats are more likely than females to continue to respond using a lever that no longer produces reinforcement (42). Also, under ratio schedules, the performance of castrated males resembles the lower response rates more typical of control females, suggesting the influence of testosterone (43). Females, on the other hand, tend to respond more efficiently than males under a DRL reinforcement schedule (41).

Materials and Methods

Subjects: breeding and exposure. We used Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Madison, WI) as subjects. On arrival at the University of Rochester Medical Vivarium, 40 females 6 weeks of age and 20 males 12 weeks of age were housed singly in polycarbonate cages in a temperature-controlled ($\pm 22^\circ\text{C}$) barrier facility provided with independent, filtered air and were maintained on a 12-hr light/12-hr dark cycle. Food and tap water were supplied *ad libitum*. Breeding began after 2 weeks of acclimation to the vivarium quarters. For breeding, two females were placed with one male overnight (approximately from 1600 to 0830 hr) in hanging wire cages. GD 0 was designated as the day on which sperm were detected in the vaginal smear obtained from each female at approximately 0830 hr; at that time, each dam was placed in a separate polycarbonate cage.

On the morning of GD 8, we assigned 36 pregnant dams to each of 3 treatment groups: 20, 60, 180 ng/kg TCDD, or a control

group, according to a randomized block design. TCDD, 98% purity (Cambridge Isotope Laboratories, Inc, Andover, MA), suspended in corn oil, was administered by gavage in the Supertox facility in the University of Rochester Environmental Health Sciences Center. For control animals, an equivalent volume of corn oil was administered.

Animal care and welfare procedures complied with National Institutes of Health guidelines. The vivarium is certified by the Association for Assessment and Accreditation of Laboratory Animal Care. Health surveillance of all animals was conducted under the direction of the Laboratory Animal Services Shared Facility of the Environmental Health Sciences Center.

Litters. Postnatal day (PND) 0 was designated as the first day on which a new litter was discovered by 0830 hr. Gestational length, number of live offspring, and sex distribution and appearance of the offspring were assessed. We recorded pup weights on PNDs 1, 4, 8, 12, 16, and 20. On PND 4, litters were culled to 5 females and 5 males, when possible. After weaning on PND 21, offspring were housed in pairs with same-sex littermates until PND 60. After PND 60, all offspring were housed individually in standard polycarbonate cages. A total of 22 healthy, appropriately distributed litters were generated from the breeding. The number of litters in dose groups assigned to control, 20, 60, and 180 ng/kg TCDD were 5, 6, 6, and 5, respectively, except for the multiple-schedule measures, where there were 5, 5, 6, and 5, respectively. Offspring were fed *ad libitum* until PND 80, at which time a fixed amount of food was supplied daily to maintain constant body weights (males, 290–330 g; females, 235–255 g) throughout the experiment. On PND 80, we randomly selected one male and one female from each litter for the current experiment.

Apparatus. Behavioral testing was conducted in 12 matched operant chambers (Model E 10-10RF; Coulbourn Instruments, LLC, Allentown, PA) containing two levers along one wall, with one active and the other not active, which will not be considered further. The levers were centered 4 cm above the floor and 12 cm apart from one another. Reinforcers, 45-mg standard lab animal diet pellets (Noyes Precision Food Pellets; Rodent Diet, P.J. Noyes Co., Inc., Lancaster, NH), were delivered to a recessed feeder receptacle mounted between the levers 8 cm above the floor. When a pellet was delivered, both the feeder light and an audible clicker were turned on for 0.5 sec. Pressing the lever with a force of 25 N or greater closed a microswitch sensed by the computer controlling the experimental events. A house

light was mounted in the center of the ceiling. The operant chambers were housed inside sound-attenuating chambers, and a fan provided ventilated air. Schedule control and data acquisition were accomplished by means of the SKED software system (State Systems, Kalamazoo, MI) run on a PDP 11/93 computer (Digital Equipment Corporation, Maynard, MA). Data were collected as interval times with a 10-msec resolution for all responses and schedule events.

Behavioral methods. We initiated the behavioral procedures (Table 1) when rats were 90 days old. Sessions were conducted once per day, 5 days per week (Monday–Friday). For both condition 1 and condition 2, each session remained in effect for 45 min or for 50 reinforcements, whichever occurred first. A 5-sec timeout (TO) started with each pellet delivery. During TO, responses to the lever were ineffective. The TO ensured that brief overruns in responding, which can occur at the time of pellet delivery, affected neither the FR nor DRL schedule consequences, and they were excluded from analyses.

Preliminary training. During preliminary training, the rats were first exposed to a concurrent variable-time 30.5-sec schedule (VT) FR 1 schedule. Under a VT schedule, a series of intervals of different durations ends with delivery of a pellet, independent of the rat's behavior. Under this concurrent schedule, a pellet was delivered whenever the rat pressed the lever once (FR 1) or the variable interval had elapsed. A session terminated after 100 pellets were delivered. This training step was completed either after two sessions in which at least 25 reinforcers had been obtained by lever pressing or after six sessions. Training then started under a FR 1 reinforcement schedule. Each rat was trained to a criterion of two successive sessions, in each of which 50 pellets were obtained. The incremental FR procedure began after all animals met this criterion.

Condition 1: incremental fixed-ratio. Responses were reinforced according to successively larger FR values in the following sequence: 1, 6, 11, 21, 31, 41, 51, 61, and 71. A new criterion was established at the beginning of every four sessions and remained constant within the sessions.

For this procedure, the dependent variables consisted of *a*) rate of FR responding: responses per session minutes; and *b*) local response rate: responses per session minutes excluding the time to the first response in a FR run of responses.

At the end of the sequence, four FR-71 extinction sessions were conducted. During those sessions, all conditions were the same as FR-71 except that the pellets were delivered to a location behind the foodcup where

the rat could not obtain the pellet. The houselight remained on throughout the session under condition 1.

Condition 2: multiple-fixed ratio 11, DRL 10 sec. The multiple schedule was introduced after the FR acquisition sequence had been completed. In this multiple schedule, FR 11 comprised one component schedule that replicated the previously studied FR 11. During the FR component, the chamber houselight remained on. A DRL 10-sec schedule comprised the second component. Under the DRL schedule, a clock began at the onset of the component and after each lever press. Only a press that occurred after the criterion interval 10 sec had elapsed was reinforced with a food pellet. During the DRL component, the chamber houselight flickered at a rate of 200 msec on and 200 msec off. Component changes occurred in strict alternation independent of responding. The FR component duration was 1 min; the DRL component duration was 5 min.

For this procedure, the dependent variables were designated as follows: *a*) rate of FR responding: FR responses per FR component minutes; *b*) local response rate: responses per session minutes excluding the time to the first response in a fixed-ratio run of responses; *c*) rate of DRL of responding: DRL responses per DRL component minutes; *d*) DRL rate of reinforcement: DRL pellets per DRL component minutes; *e*) proportion of DRL responses reinforced; and *f*) FR relative rate of responding: the ratio of FR responses per FR component minutes to total responses

Table 1. Number of sessions conducted for the incremental fixed ratio and mult-FR 11, DRL 10-sec schedules.

Value	Number of sessions
Preliminary training	
VT + FR 1	2
FR 1	3–4
Incremental FR	
1	4
6	4
11	4
21	4
31	4
41	4
51	4
61	4
71	4
Retrain	
VT + FR 1	1
FR 11	2
Extinction	
FR 11	4
Retrain	
VT + FR 1	1
FR 11	2
Multiple	
FR 11 DRL 10 sec	30
Extinction	
FR 11 DRL 10 sec	2

per session minutes, which served as an index of schedule discrimination

Statistical methods. The General Linear Model procedure (44) was used to examine the behavioral data, primarily by repeated-measures analysis of variance (ANOVA; using SAS version 8, SAS Institute, Cary, NC). Prenatal treatment was the between-subject factor. Because one male and one female littermate were drawn from each litter, the statistical unit of analysis was litter, with sex included as a within-subject factor. For the incremental FR reinforcement schedule, four sessions at each of the nine FR values were treated as within-subject factors for repeated measurements. For the multiple FR 11 DRL 10 sec reinforcement schedule, the six dependent variables were analyzed separately. For each variable, the data were averaged over five consecutive

sessions (six blocks) preceding the ANOVA, which included the factors sex, treatments, and blocks (the last being repeated measurements). To evaluate the dose by sex interaction, the data of the male and female offspring were collapsed across the six blocks. We then analyzed these data for linear and quadratic contrasts between sexes. For both incremental FR and multi-FR DRL reinforcement schedules, we used the Huynh-Feldt (45) adjustment to the degrees of freedom when appropriate. For the multiple schedule, we used a mixed procedure to evaluate local FR responses/minute because not all animals responded under the FR schedule at sufficient levels to evaluate complete sets of male–female littermate pairs.

Benchmark dose analysis. Dose–response relationships were described by benchmark dose modeling software, version 1.3, provided

by the U.S. Environmental Protection Agency (BMDS, U.S. EPA, Research Triangle Park, NC). The benchmark approach (46) is a useful alternative to the more traditional no-observed-adverse-effect level calculations used to derive exposure standards. Benchmark calculations consider the entire dose–response relationship and do not involve extrapolations far below experimental observations. The benchmarks we calculated represent doses that are associated with specific operant behavior performance. With the continuous model, we calculated benchmark doses representing the model-estimated control mean minus proportional deviations equivalent to a 10% (ED_{10}) or 1% (ED_{01}) change. The BMDS software also provides a 95% lower bound that can be divided by a standard uncertainty factor, such as 100 to calculate a reference dose or provide a margin of exposure.

Results

Maternal and postpartum data. All dams delivered within 3 weeks after determination of pregnancy. The group mean weight gain across the gestational period, shown in Table 2, ranged from 68 to 79 g. The number of

Table 2. Mean \pm SD dam body weights across the gestational period.

Dose (ng/kg)	Body weight (g) on gestation day				
	0	4	8	12	16
Control	270.83 \pm 14.11	306.00 \pm 5.66	298.33 \pm 18.99	311.67 \pm 22.11	346.83 \pm 18.05
20	275.00 \pm 19.62	299.33 \pm 13.95	302.00 \pm 18.67	321.50 \pm 19.68	353.75 \pm 27.01
60	262.00 \pm 22.29	275.50 \pm 24.08	288.29 \pm 22.19	306.00 \pm 18.58	337.57 \pm 21.60
180	278.00 \pm 16.40	292.86 \pm 17.54	304.29 \pm 20.99	320.57 \pm 17.65	346.00 \pm 21.29

Table 3. Mean \pm SD pup sex distribution and weight gain across the lactational period.

Dose group (ng/kg)	Pups/litter	Weight gain (g) on postnatal day				
		4	8	12	16	20
Male						
Control	6.43 ± 2.07	11.26 ± 2.39	20.01 ± 3.02	30.07 ± 3.63	38.68 ± 4.67	53.81 ± 6.93
20	6.88 ± 2.10	11.22 ± 1.22	20.26 ± 2.81	33.28 ± 4.73	42.22 ± 3.47	56.73 ± 4.86
60	7.29 ± 2.75	11.02 ± 1.11	20.23 ± 1.96	30.01 ± 2.15	40.65 ± 2.66	54.77 ± 4.58
180	5.71 ± 3.55	11.49 ± 1.70	21.84 ± 2.72	31.39 ± 3.07	41.75 ± 3.44	55.31 ± 5.10
Female						
Control	5.71 ± 2.29	11.04 ± 2.58	19.67 ± 3.28	28.44 ± 5.08	38.23 ± 4.96	53.14 ± 7.14
20	6.88 ± 2.80	10.57 ± 1.07	19.34 ± 1.93	30.11 ± 2.87	39.59 ± 2.84	54.19 ± 4.63
60	5.14 ± 1.57	10.49 ± 1.26	19.62 ± 2.07	29.43 ± 1.82	39.53 ± 2.47	53.77 ± 4.17
180	5.29 ± 1.60	11.42 ± 1.50	21.63 ± 2.43	31.80 ± 3.96	40.98 ± 6.46	55.65 ± 5.60

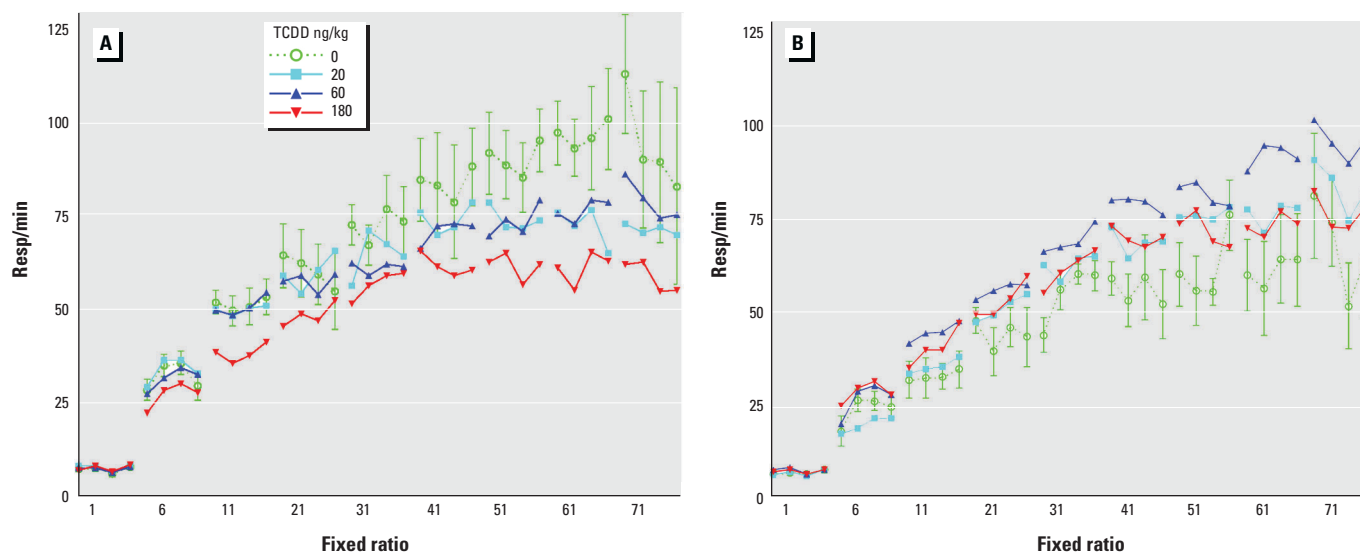


Figure 1. Mean (\pm SEM for controls) rate of responding per session for the four TCDD exposure groups during the incremental fixed-ratio condition: (A) males; (B) females. Resp, responding.

male and female pups per litter and their body weights are summarized in Table 3. None of those observations indicated an effect of exposure.

Behavioral data. All animals acquired the lever-press response within 3 or 4 days of preliminary training.

Incremental fixed-ratio condition. Mean response rates (responses/min) for each FR value are shown in Figure 1. The ANOVA evaluated the contribution of TCDD treatment, and also several interactions including treatment by sex and treatment by sex by FR value. None of those results were statistically significant. We also examined the local rate of responding (i.e., the rate of responding corrected for the postreinforcement pause; data not shown), and similarly observed no significant effects.

Multiple FR 11, DRL 10-sec reinforcement schedule. TCDD treatment affected almost all of the variables studied. The ANOVA results showed that although neither main exposure nor sex effects per se were seen, interactions were observed for every response measure except FR relative rate (Table 4). These significant results are examined below.

FR component. Mean response rates of the males and females across blocks of five sessions during the FR component are shown in Figure 2. For the males, all three groups exposed to TCDD responded at lower rates than the controls. For the females, all three TCDD-treated groups responded at higher rates than controls. The significant treatment-by-sex interaction ($p = 0.036$) for FR response rate is depicted in Figure 3, which plots the mean response rates of males and females collapsed across session blocks. Although the mean rate for control males exceeded that for control females, this relationship changed across doses. For example, the 60 ng/kg females responded at higher rates than did the 60 ng/kg males. The ANOVA of sex differences in FR response rate revealed a significant quadratic trend ($p = 0.01$).

This TCDD prenatal treatment-by-sex interaction was examined further with benchmark dose analyses. The fitted polynomial (Figure 4) was based on the following data: For each of the six blocks of five sessions each, the mean response rate of a female was subtracted from the mean response rate of its male littermate. Those littermate, male–female differences were then averaged across the six blocks to yield a mean difference for each litter within each dose group. The means and standard deviations of those data (Table 5) complied with a second-order polynomial function ($p = 0.01$), as seen in Figure 4. With the BMDS continuous model, we calculated the ED_{10} or ED_{01} as well as a 95% lower bound (Table 6). The ED_{10} for FR response rate was 2.77 ng/kg, with a 95% lower bound of 1.81 ng/kg.

DRL component. Mean response rates of male and female offspring across blocks of five sessions during the DRL 10-sec component are shown in Figure 5. As with the FR component, the ANOVA indicated a significant sex-by-treatment interaction ($p = 0.01$). Duplicating the FR analysis, all three male dose groups responded at lower rates than controls. For the females, all three TCDD-treated groups responded at higher rates than controls. The interaction is seen in Figure 6, which shows the mean response rates of both males and the females collapsed

across session blocks. The mean rate for control males exceeded that for control females, but this relation changed across doses (e.g., the 60 ng/kg females responded at higher rates than did the 60 ng/kg males). The ANOVA analysis of the sex difference in DRL response rate again revealed a significant quadratic trend ($p = 0.01$).

A benchmark dose analysis of the treatment-by-sex interaction in DRL response rate, based on the data in Table 5 as for FR response rate, showed it to be accurately modeled by a second-order polynomial ($p = 0.01$), as seen in Figure 7. As shown in Table 6, the ED_{10} was 2.97 ng/kg, with a 95% lower bound of 2.02 ng/kg. The ED_{01} was 0.30 ng/kg, with a 95% lower bound of 0.20 ng/kg.

Response rate under an FR schedule directly controls the rate of reinforcement. Under the DRL schedule, however, identical rates of responding need not produce identical rates of reinforcement because reinforcement depends on the distribution of responses across time. Efficiency of responding, (i.e., the ratio of reinforced responses to total responses), shown in Figure 8, measures how precisely responding meets the DRL criterion. It indicates that control females responded more efficiently than control males. The plot also depicts the nature of the sex-by-treatment interaction shown in

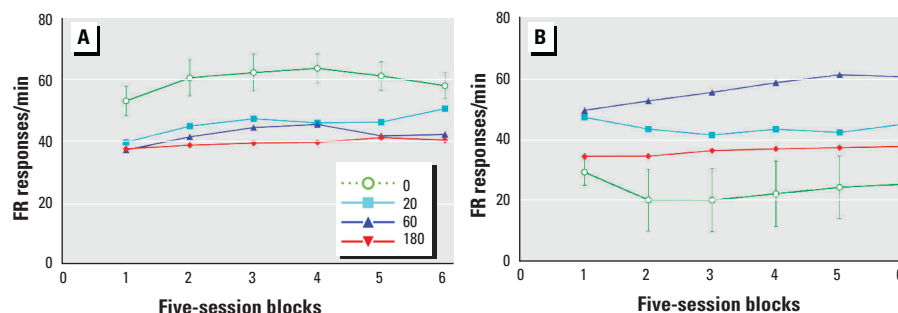


Figure 2. Mean (\pm SEM for controls) rate of responding across the six 5-session blocks for the four TCDD exposure groups during the fixed-ratio component of the mult-FR 11, DRL 10-sec condition: (A) males; (B) females.

Table 4. Results from the general linear models procedure, repeated-measures ANOVA: factor, degrees of freedom, and p -values for the mult-FR 11, DRL 10-sec response measures.

Factor	df	FR responses/min	DRL responses/min	DRL reinforcements/ DRL responses	FR relative rate	DRL reinforcements/min	Local FR responses/min ^a
TCDD Dose (treatment)	3, 17	0.67	0.43	0.37	0.63	0.59	0.69
Sex	1, 17	0.25	0.20	0.03	0.21	0.07	0.52
Sex \times treatment	3, 17	0.04	0.01	0.09	0.40	0.03	0.04
Block	5, 85	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Block \times treatment	15, 85	0.49	0.58	0.02	0.33	0.18	0.86
Sex \times block	5, 85	0.07	0.16	< 0.01	0.46	0.27	0.21
Sex \times block \times treatment	15, 85	0.28	0.27	0.14	0.36	0.30	0.91
Linear trend: sex difference (M – F)	1	0.23	0.09	0.20	0.73	0.20	No analysis
Quadratic trend: sex difference (M – F)	1	0.01	0.01	0.03	0.10	0.01	No analysis

Abbreviations: df, degrees of freedom; F, female; M, male. The Huynh-Feldt correction is indicated where block is a factor, except for the local FR responses/min.

^aNo analysis if < 5 ratios were completed.

Table 4. The ANOVA analysis of the sex difference in DRL efficiency showed a significant quadratic trend ($p = 0.03$).

The ANOVA contrast of the sex difference across treatments was also conducted for two other measures. DRL reinforcements per minute showed a significant quadratic trend ($p = 0.01$); the mean of the sex difference across doses in Table 5 shows the nature of the trend for this measure. The contrast of sex difference was not significant for FR relative rate ($p = 0.10$).

Extinction. Examination of the graphical data did not suggest any effects of treatment on performances during the four extinction sessions after the incremental FR procedure or the two sessions after the mult-FR 11

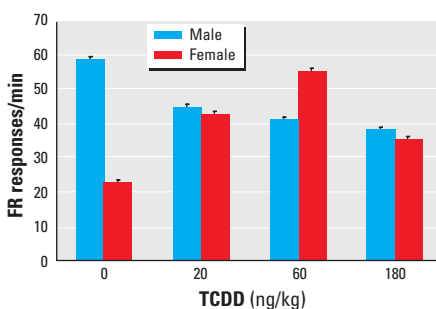


Figure 3. Mean (\pm SEM) response rate for male and female littermates during the fixed-ratio component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group.

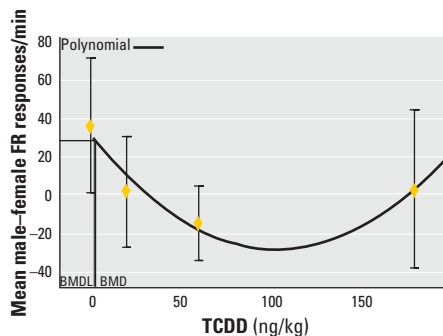


Figure 4. Polynomial model for benchmark dose ED_{10} value and 95% lower confidence level for the male-female littermate differences in FR response rate during the fixed-ratio component of the mult-FR 11, DRL 10-sec schedule. The polynomial was calculated from a quadratic fit to the dose-effect data shown for FR responses/min in Table 5.

Table 5. Male-female littermate differences for each response measure for mult-FR 11, DRL 10-sec.

Dose (ng/kg)	No.	FR responses/min		DRL responses/min		DRL reinforcements/ DRL responses		FR relative rate		DRL reinforcements/min	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	5	36.113	28.051	18.436	7.986	-0.182	0.093	0.079	0.145	-0.814	0.448
20	5	1.887	23.014	-0.987	10.963	-0.115	0.209	0.044	0.111	-0.364	0.821
60	6	-14.301	18.143	-4.522	7.194	0.048	0.081	-0.035	0.046	0.374	0.540
180	5	3.113	32.917	-0.406	15.231	-0.042	0.166	0.039	0.127	-0.163	0.443

For each of the six blocks of five sessions each, the mean response rate of a female was subtracted from the mean response rate of its male littermate. Those littermate, male-female differences were then averaged across the six blocks to yield a mean difference for each litter within each dose group. Littermate differences were then averaged across the number of litters for each dose of TCDD.

DRL 10-sec reinforcement schedule (data not shown). No further analyses of those data were conducted.

Discussion

Administration of TCDD on GD 8 to pregnant rats altered the schedule-controlled performance of their offspring. The most striking result is the sexually dimorphic pattern of responses. This pattern was seen most clearly under the mult-FR DRL schedule. Figure 1 indicates a similar pattern of sex differences under the incremental FR schedule. Under both conditions, TCDD-exposed males responded at lower rates than control males. Females displayed an opposite pattern, with TCDD exposure associated with higher rates.

When the multiple schedule was introduced, conditions during the FR component replicated those of the incremental FR 11 condition. The DRL component, however, offered a marked contrast in response requirements and stimulus conditions. In particular, while the FR contingency selectively reinforced short inter-response times (IRTs) and high rates of lever pressing, the DRL contingency selectively reinforced long IRTs and low rates of lever pressing. The FR relative rate measure (see Table 5) describes how well the subjects discriminated between

the response requirements of the two component schedules. This index did not differ among groups, indicating that under those specific conditions TCDD did not affect acquisition of the discrimination.

Sexually dimorphic patterns of responding have been observed in many schedule-controlled operant behaviors. For example, under a random ratio schedule, which generally maintains high rates of responding, males respond at higher rates than females. Under DRL schedules, females generally perform more efficiently than males (38,47). Similar response patterns were also observed in control offspring in the present experiment. Such behavioral differences between the sexes appear not to be a function of sex differences in food motivation. Instead, they are influenced at least partly by the presence or absence of gonadal hormones, specifically the male gonadal hormone testosterone (48). These response patterns can be altered by external hormonal exposure (38).

Although we did not directly measure gonadal function in the offspring, our data support a role for TCDD-induced alterations in neuroendocrine function. Previous studies have repeatedly reported that TCDD, even at relatively low doses, interferes with normal development of reproductive function, including sex-specific patterns of reproductive

Table 6. BMDs and 95% lower bound (95% LB) calculations based on a 1% or a 10% shift from the control group mean (ED_{01} or ED_{10}) for mult-FR DRL response measures.

	FR responses/min		DRL responses/min	
	ED_{01}	ED_{10}	ED_{01}	ED_{10}
BMD (ng/kg)	0.27	2.77	0.30	2.97
95% LB	0.18	1.81	0.20	2.02

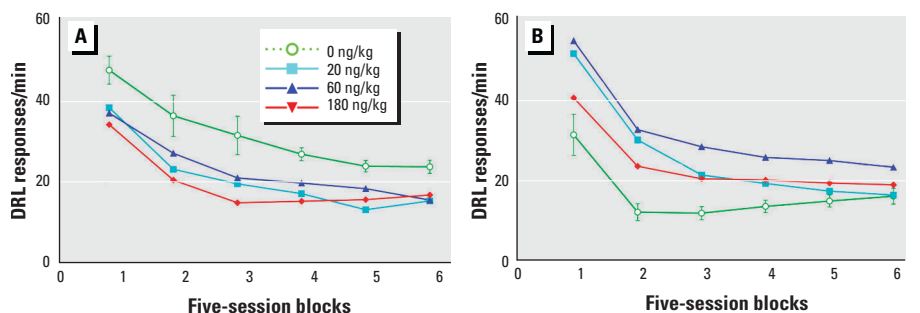


Figure 5. Mean (\pm SEM for controls) rate of responding across the six 5-session blocks for the four TCDD exposure groups during the DRL component of the mult-FR 11, DRL 10-sec condition: (A) males; (B) females.

behavior (49–52). The remarkable differences between male and female offspring in how TCDD affected operant behavior strongly suggest that its influence is exerted through the effect of gonadal hormones on brain development. In addition, the current results provide an intriguing counterpoint to those of our recent study (14), in which TCDD was administered at doses of 0, 20, 60, and 180 ng/kg to pregnant dams on GD 18. The earlier experiment tested the performance of only the female offspring in a situation in which the subjects responded on FR schedules for the opportunity to exercise in a running wheel. In that experiment, TCDD produced significant dose-related reductions in performance. The results of the current study, coupled with the findings of the previous study, emphasize the need for further investigation of how TCDD modifies the course of brain development, especially in relation to the markers of sexual differentiation. In rats, markers of sexual differentiation appear late in gestation (53).

The male–female differences in response to prenatal TCDD exposure followed a U-shaped function across the doses studied. This outcome is not unique. It is becoming

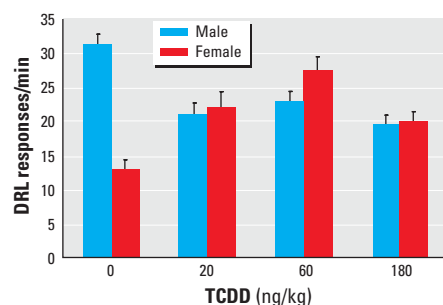


Figure 6. Mean (\pm SEM) response rate for male and female littermates during the DRL component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group.

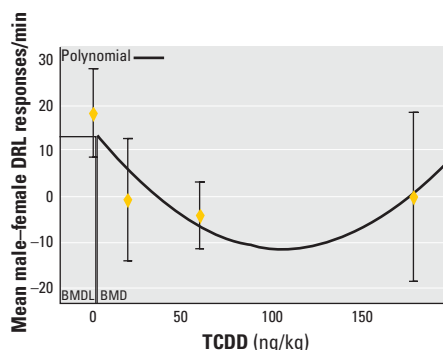


Figure 7. Polynomial model for benchmark dose ED_{10} value and 95% lower confidence level for the male–female littermate differences in DRL response rate during the DRL component of the mult-FR 11, DRL 10-sec schedule. The polynomial was calculated from a quadratic fit to the dose–effect data shown for DRL responses/min in Table 5.

increasingly recognized that, especially for endocrine-disrupting agents, monotonic dose–response functions may not be the prevalent pattern (54,55). Similar results were reported in our previous study in which rats were exposed to TCDD doses of 0, 60, 180 and 540 ng/kg on GD 15 (56). On a delayed visual discrimination task, the performance of both male and female offspring exposed to 180 ng/kg TCDD was significantly less accurate than the lowest and the highest exposure dose groups. Seo et al. (13) also observed U-shaped dose–effect functions. Male offspring exposed to a total dose of 700 ng/kg made significantly fewer errors in a radial arm maze, but males exposed to 1,400 ng/kg resembled controls. Moreover, vom Saal et al. (55,57) reported that perinatal exposure to estradiol and diethylstilbestrol (DES) increased prostate weight in rats described by an inverted-U relationship between dose and response. Prostate weight changed in response to the medium dose of estradiol or DES but did not react to the highest dose of estradiol or DES.

Generally, testing methods for systemic toxicants, which include endocrine disruptors, are based on the assumption of a monotonic dose relationship, where the response to an environmental chemical is assumed to increase as dose increases. Results from our experiments and others just noted reliably demonstrate a curvilinear response to dose. Curvilinear dose–response functions such as those seen in the hormesis literature (58–60) are difficult to explain in our case because of our limited understanding of the toxic mechanisms underlying perinatal TCDD exposure.

Whatever the mechanisms, the current findings, especially the benchmark dose analyses, indicate that current human body burdens based on TEQs, even though they may have fallen since 1995 (5), may represent a health hazard. Human data on the developmental

neurotoxicity of this class of compounds are almost totally absent except for studies linking PCBs and impaired child development (61,62). Reductions of exposure, coupled with further research on the behavioral mechanisms and consequences of exposure to this class of chemicals, including studies of brain structure (63), are clearly warranted.

REFERENCES AND NOTES

- Gray LE, Kelce WR, Monosson E, Ostby JS, Birnbaum LS. Exposure to TCDD during development permanently alters reproductive function in male Long-Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol Appl Pharmacol* 131:108–118 (1995).
- Pohjanvirta R, Viluksela M, Tuomisto JT, Unkila M, Karasinska J, Franc MA, Holowenko M, Giannone JV, Harper PA, Tuomisto J, et al. Physicochemical differences in the Ah receptors of the most TCDD susceptible and the most TCDD resistant rat strains. *Toxicol Appl Pharmacol* 155:82–95 (1999).
- Nau H, Bass R, Neubert D. Transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) via placenta and milk, and postnatal toxicity in the mouse. *Arch Toxicol* 59:36–40 (1986).
- Schantz SL, van Valkenberg HC, Bowman RE. Effects of previous maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on social behavior of rhesus monkey mother–infant pairs [Abstract]. *Teratology* 31(3):B–9 (1985).
- DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ Health Perspect* 103:820–831 (1995).
- Abbott BD, Schmid JE, Pitt JA, Buckalew AR, Wood CR, Held GA, Dilberto JJ. Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. *Toxicol Appl Pharmacol* 155:62–70 (1999).
- Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. *Drug Metab Dispos* 26:1194–1198 (1998).
- Gray LE, Ostby J, Goldman J. Methoxychlor-induced alterations of estrogen-dependent running wheel activity, the reproductive tract and pituitary function in the female rats. *Toxicol Appl Pharmacol* 96:525–540 (1988).
- Sewall CH, Bell DA, Clark GC, Tritscher AM, Tully DB, Vanden Heuvel J, Lucier GW. Induced gene transcription: implications for biomarkers. *Clin Chem* 41:1829–1834 (1995).
- Schantz SL, Bowman RE. Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* 11:13–19 (1989).
- Schantz SL, Seo B-W, Moshtaghian J, Peterson RE, Moore RW. Effects of gestational and lactational exposure to TCDD or coplanar PCBs on spatial learning. *Neurotoxicol Teratol* 18:305–313 (1996).
- Seo B-W, Sparks AJ, Medora K, Amin S, Schantz SL. Learning and memory in rats gestationally and lactationally exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* 21:231–239 (1999).
- Seo B-W, Powers B, Widholm J, Schantz SL. Radial arm maze performance in rats following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* 22:511–519 (2000).
- Markowski VP, Zareba G, Stern S, Cox C, Weiss B. Altered operant responding for motor reinforcement and the determination of benchmark doses following perinatal exposure to low-level 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 109:621–627 (2001).
- Weiss B, Cory-Slechta DA. Assessment of behavioral toxicity. In: *Principles and Methods of Toxicology* (Hayes AW, ed). 4th ed. Philadelphia:Taylor & Francis, 2001;1451–1528.
- van Haaren F, Haworth SC, Bennett SM, Cody BA, Hoy JB, Karlis JL, Tebbett IR. The effects of pyridostigmine bromide, permethrin, and DEET alone, or in combination, on fixed-ratio and fixed-interval behavior in male and female rats. *Pharmacol Biochem Behav* 69:23–33 (2001).

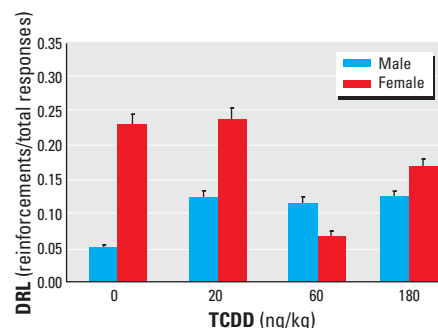


Figure 8. Mean (\pm SEM) DRL efficiency (DRL reinforcements/DRL responses) for male and female littermates during the DRL component for the 30 sessions of the mult-FR 11, DRL 10-sec condition for each TCDD exposure group. A value of 1 indicates that each DRL response was reinforced with a pellet delivery, and a value of 0 indicates that no DRL response was reinforced.

17. Rice DC, Hayward S. Effects of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation behavior (concurrent random interval-random interval and progressive ratio performance) in rats. *Neurotoxicol Teratol* 21:679-687 (1999).
18. Newland MC, Rasmussen EB. Aging unmasks adverse effects of gestation exposure to methylmercury in rats. *Neurotoxicol Teratol* 22:819-828 (2000).
19. Altman J, Bayer SA. *Atlas of Prenatal Rat Brain Development*. Boca Raton, FL: CRC Press, 1995.
20. Abbott BD, Birnbaum LS, Perdev GH. Developmental expression of two members of a new class of transcription factors. I. Expression of aryl hydrocarbon receptor in the C57BL/6N mouse embryo. *Dev Dyn* 204:133-143 (1995).
21. Abbott BD, Probst MR. Developmental expression of two members of a new class of transcription factors. II. Expression of aryl hydrocarbon receptor nuclear translocator in the C57BL/6N mouse embryo. *Dev Dyn* 204:144-155 (1995).
22. Hurst CH, Abbott BD, DeVito MJ, Birnbaum LS. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in pregnant Long Evans rats: disposition to maternal and embryo/fetal tissues. *Toxicol Sci* 45:129-136 (1998).
23. Sidman M. *Tactics of Scientific Research: Evaluating Experimental Data in Psychology*. New York: Basic Books, 1960.
24. Weiss B. The fine structure of behavior during transition states. In: *The Theory of Reinforcement Schedules* (Schoenfeld WN, ed). New York: Appleton-Century-Crofts, 1970:277-311.
25. Gentry GD, Middaugh LD. Prenatal ethanol weakens the efficacy of reinforcers for adult mice. *Teratology* 37:135-144 (1988).
26. Gentry GD, Middaugh LD. Using the development of fixed-ratio schedule control to detect long-term effects of drugs and toxicants. In: *Neurobehavioral Toxicity* (O'Donoghue JL, Weiss B, eds). New York: Raven, 1994:319-330.
27. Newland MC, Yezhou S, Logdberg B, Berlin M. Prolonged behavioral effects of in utero exposure to lead or methylmercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicol Appl Pharmacol* 126:6-15 (1994).
28. Newland MC, Warfvinge K, Berlin M. Behavioral consequences of in utero exposure to mercury vapor: alterations in lever-press durations and learning in squirrel monkeys. *Toxicol Appl Pharmacol* 139:374-386 (1996).
29. Newland MC, Ng WW, Baggs RB, Gentry GD, Weiss B, Miller RK. Operant behavior in transition reflects neonatal exposure to cadmium. *Teratology* 34:231-241 (1986).
30. Bushnell PJ, Bowman RE. Persistence of impaired reversal learning in young monkeys exposed to low levels of dietary lead. *J Toxicol Environ Health* 5:1015-1023 (1979).
31. Bushnell PJ, Bowman RE. Effect of chronic lead ingestion on social development in infant rhesus monkeys. *Neurobehav Toxicol* 1:207-219 (1979).
32. Hughes JA, Sparber SB. d-Amphetamine unmasks postnatal consequences of exposure to methylmercury in utero: methods for studying behavioral teratogenesis. *Pharmacol Biochem Behav* 8:365-375 (1978).
33. Weiss B, Reuhl K. Delayed neurotoxicity: a silent toxicity. In: *Principles of Neurotoxicology* (Chang LW, ed). New York: Dekker, 1994:765-781.
34. Spear LP. Assessment of the effects of developmental toxicants: pharmacological and stress vulnerability of offspring. *NIDA Res Monogr* 164:125-145 (1996).
35. Rice DC, Gilbert SG. Low lead exposure from birth produces behavioral toxicity (DRL) in monkeys. *Toxicol Appl Pharmacol* 80:421-426 (1985).
36. Wood RW, Rees DC, Laties VG. Behavioral effects of toluene are modulated by stimulus control. *Toxicol Appl Pharmacol* 68:462-472 (1983).
37. Cohn J, Cory-Slechta DA. Lead exposure potentiates the effects of NMDA on repeated learning. *Neurotoxicol Teratol* 16:455-465 (1994).
38. Beatty WW, Gregoire KC, Parmar LL. Sex differences in retention of passive avoidance behavior. *Bull Psychon Soc* 2:99-100 (1973).
39. Heinsbroek RPW, van Haaren F, Zantvoord F, van de Poll NE. Sex differences in response rates during random-ratio acquisition: effects of gonadectomy. *Physiol Behav* 39:269-272 (1987).
40. van Haaren F, Heinsbroek RPW, Louwerse A, van de Poll NE. d-Amphetamine differentially affects low, but not high response rates of male and female Wistar rats. *Psychopharmacology (Berl)* 89(1):73-76 (1986).
41. van Hest A, van Haaren F, van de Poll NE. Behavioral differences between male and female Wistar rats in food rewarded lever holding. *Physiol Behav* 39:263-267 (1987).
42. van Hest A, van Haaren F, van de Poll NE. Perseverative responding in male and female Wistar rats: effects of gonadal hormones. *Horm Behav* 23:57-67 (1989).
43. Heinsbroek RPW, van Haaren F, Zantvoord F, van de Poll NE. Effects of pentobarbital and progesterone on random ratio responding in male and female rats. *Psychopharmacol* 93:178-181 (1987).
44. McCullagh P, Nelder JA. *Generalized Linear Models*. 2nd ed. London: Chapman and Hall, 1989.
45. Crowder MJ, Hand DJ. *Analysis of Repeated Measures*. London: Chapman and Hall, 1990.
46. Crump KS. A new method for determining allowable daily intakes. *Fundam Appl Toxicol* 4:854-871 (1984).
47. Kearly RC, van Hartesveldt C, Woodruff ML. Behavioral and hormonal effects of hippocampal lesions on male and female rat. *Physiol Psychol* 2:187-196 (1974).
48. van Hest A, van Haaren F, van de Poll NE. The behavior of male and female Wistar rats pressing lever for food is not affected by sex differences in food motivation. *Behav Brain Res* 27:215-221 (1988).
49. Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol* 114:118-126 (1992).
50. Mably TA, Moore RW, Goy RW, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. *Toxicol Appl Pharmacol* 114:108-117 (1992).
51. Mably TA, Moore RW, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1. Effects on androgenic status. *Toxicol Appl Pharmacol* 114:97-107 (1992).
52. Bjerke DL, Peterson RE. Reproductive toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male rats: different effects of in utero versus lactational exposure. *Toxicol Appl Pharmacol* 127:241-249 (1994).
53. Breedlove SM. Sexual differentiation of the brain and behavior. In: *Behavioral Endocrinology* (Becker JB, Breedlove SM, Crews D, eds). Cambridge, MA: MIT Press, 1992:39-68.
54. Hattis D, Glowa J, Tilson H, Ulbrich B. Risk assessment for neurobehavioral toxicity: SGOMSEC Joint Report. *Environ Health Perspect* 104(suppl 2):217-226 (1996).
55. vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 94:2056-2061 (1997).
56. Markowski VP, Cox C, Preston R, Weiss B. Impaired response efficiency in an operant visual discrimination procedure following prenatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* (in press).
57. vom Saal FS, Nagel SC, Palanza P, Boechler M, Parmigiani S, Welshons WV. Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behavior in male mice. *Toxicol Lett* 77:343-350 (1995).
58. Davis JM, Svendsgaard DJ. U-shaped dose-response curves: their occurrence and implications for risk assessment. *J Toxicol Environ Health* 30:71-83 (1990).
59. Calabrese EJ, Baldwin LA. U-shaped dose-responses in biology, toxicology, and public health. *Annu Rev Public Health* 22:15-33 (2001).
60. Calabrese EJ, Baldwin LA. The frequency of u-shaped dose responses in the toxicological literature. *Toxicol Sci* 62:330-338 (2001).
61. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med* 335(11):783-789 (1996).
62. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J Pediatr* 134:33-41 (1999).
63. Zareba G, Hojo R, Zareba GM, Watanabe C, Markowski VP, Baggs RB, Weiss B. Sexually dimorphic alterations of brain cortical dominance in rats prenatally exposed to TCDD. *J Appl Toxicol* (in press).